Interferon Regulatory Factor 5 (IRF5) Interacts with the Translation Initiation Complex and Promotes mRNA Translation During the Integrated Stress Response to Amino Acid Deprivation

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SUPPLEMENTAL INFORMATION

Supplemental information consists of one table and six figures.

Table S1. mRNA translation-related proteins co-immunoprecipitated with IRF5

Accession No.	Protein Name	Mascot Score	Coverage (%)
4504445	HNRNP A1 (isoform a)	425	22
4504447	HNRNP A2/B1 (isoform A2)	158, 484	7, 26
14043072	HNRNP A2/B1 (isoform B1)	854	38
88963249	HNRNP A3 (isoform 1)	540	25
4758544	HNRNP C (isoform b)	403	23
14165435	HNRNP K (isoform b)	164, 495	5, 20
74136883	HNRNP U (isoform a)	426, 586	9, 16
14141161	HNRNP U (isoform b)	325	7
24234747	ILF2	211	6
4826998	SFPQ	388	14
15055539	RPS2	296	24
15718687	RPS3	409	29
4506725	RPS4X	731	41
4503471	EEF1α1	363, 457, 527	17, 20, 21
4503475	EEF1α2	257, 258, 291	11, 11, 11
25453474	EEF1δ	140	7
4503483	EEF2	345	10
4758138	DDX5	218	5
100913206	DHX9	452	7
4503529	EIF4A1	282	12

Combined data from n=3 independent experiments. Abbreviations: HNRNP=heterogeneous nuclear ribonucleoprotein; ILF=interleukin enhancer-binding factor; SFPQ=splicing factor, proline- and glutamine-rich; RPS=ribosomal protein S; EEF=eukaryotic elongation factor; DDX=DEAD-box; DHX=DEAH-box; EIF=eukaryotic initiation factor

Figure S1.

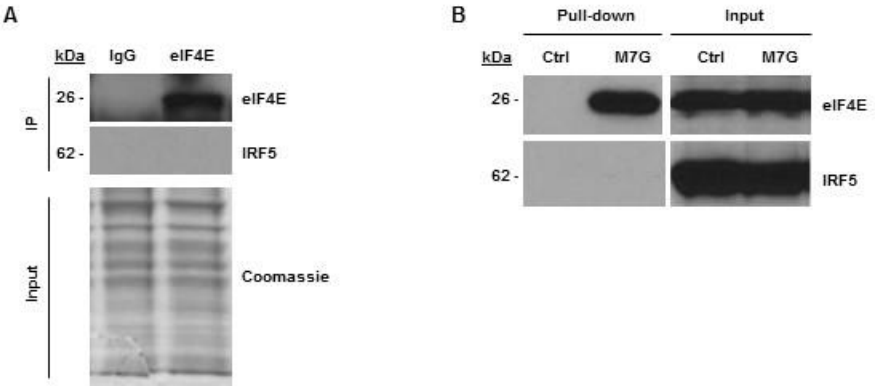

Figure S1. IRF5 does not interact with the m^{7}GTP cap-binding protein, eIF4E. A, Endogenous eIF4E was immunoprecipitated from BJAB cell lysates previously fixed with 1% formaldehyde and analyzed by immunoblotting for IRF5 co-immunoprecipitation. Results are representative of n=3 independent experiments. **B**, Cell lysates were subjected to m^{7}GTP sepharose pull-down, and analyzed by immunoblotting as indicated. Results are representative of n=3 independent experiments

Figure S2.

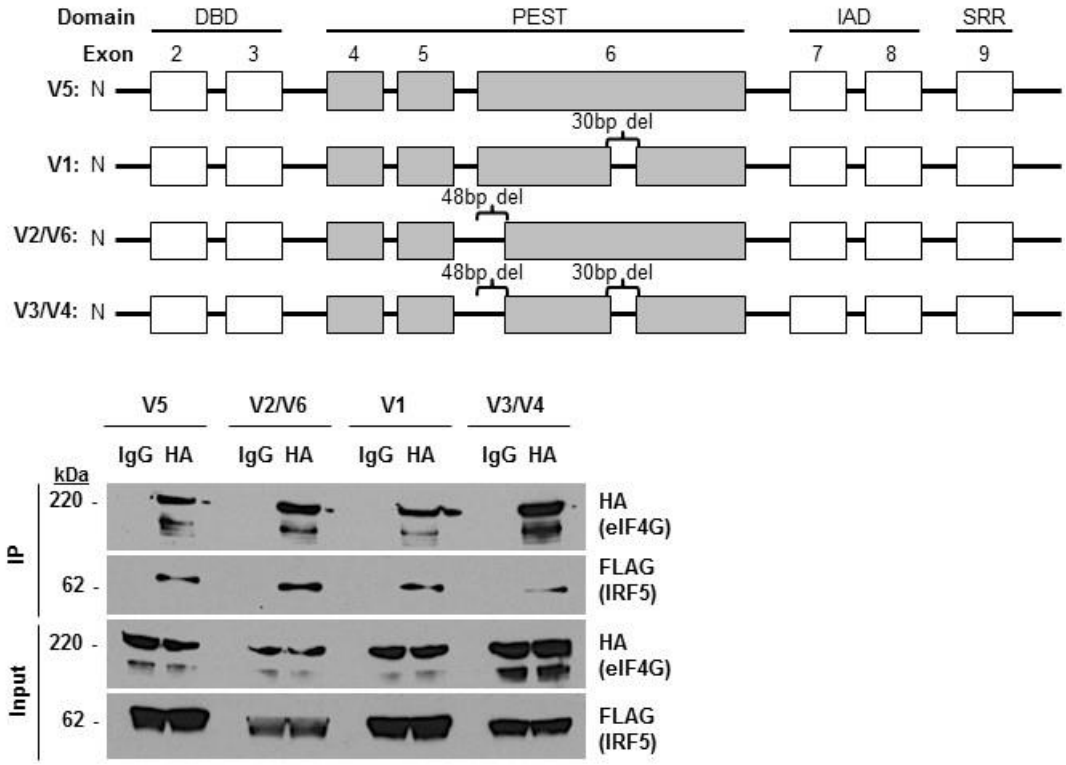

Figure S2. eIF4G interaction is unaffected by structural variability within IRF5 exon 6. **A**, Diagram representing the coding-variants in exon 6 that affect IRF5 protein structure. **B**, HEK293T cells were co-transfected with HA-tagged eIF4G and indicated FLAG-tagged IRF5 isoforms. FLAG-IRF5 isoforms were co-immunoprecipitated with HA-eIF4G and analyzed by immunoblotting. Results are representative of n=3 independent experiments.

Figure S3.

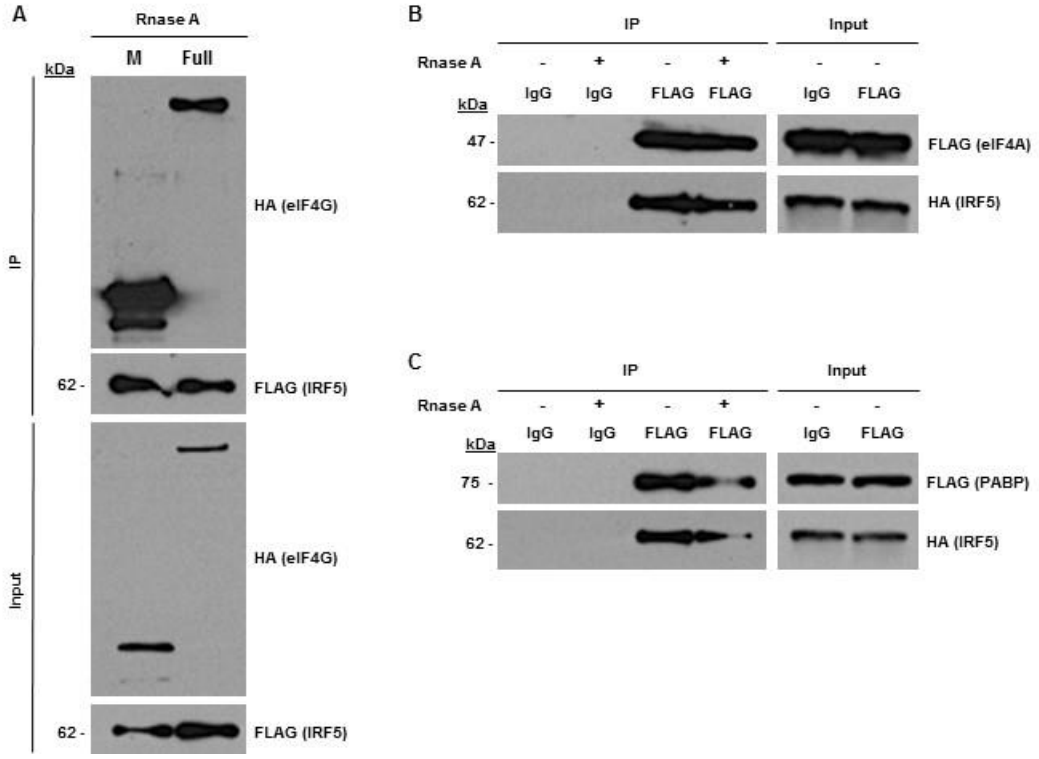

Figure S3. IRF5-TIC interaction is not RNA-dependent. **A**, HEK293T cells were co-transfected with FLAG-tagged IRF5 (V5) and HA-tagged full-length eIF4G or HA-tagged eIF4G mutant containing the MIF4G domain. Full-length HA-eIF4G or HA-MIF4G fragment was co-immunoprecipitated with FLAG-IRF5 (V5) as indicated. Individual immunoprecipitation reactions were divided equally and incubated with or without 10 μg/mL RNase A at 37°C for 10 min prior to elution. **B** and **C**, HEK293T were co-transfected with HA-IRF5 (V5) and FLAG-eIF4A (B) or FLAG-PABP (C). FLAG-eIF4A (B) or FLAG-PABP (C) were then immunoprecipitated as indicated. Individual immunoprecipitation reactions were divided equally and incubated with or without 10 μg/mL RNase A at 37°C for 10 min prior to elution. Immunoblotting was performed as indicated. (A-C) All results are representative of n=3 independent experiments.

Figure S4.

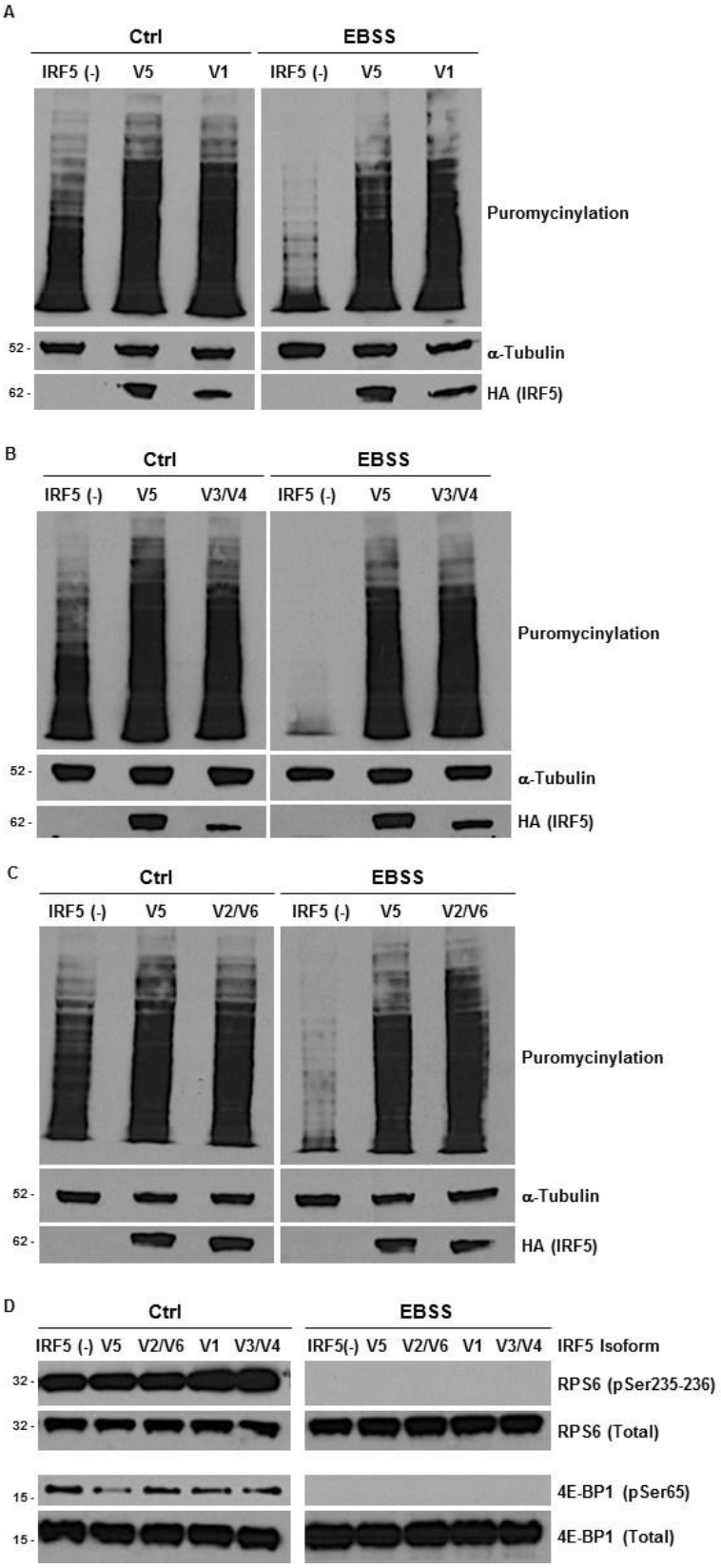
A
Ctrl
EBSS
IRF5 (-)
V5
V1
Puromycinylation
52 -
α-Tubulin
62 -
HA (IRF5)
B
Ctrl
EBSS
IRF5 (-)
V5
V3/V4
Puromycinylation
52 -
α-Tubulin
62 -
HA (IRF5)
C
Ctrl
EBSS
IRF5 (-)
V5
V2/V6
Puromycinylation
52 -
α-Tubulin
62 -
HA (IRF5)
D
Ctrl
EBSS
IRF5 (-) V5 V2/V6 V1 V3/V4
IRF5(-) V5 V2/V6 V1 V3/V4
IRF5 Isoform
32 -
RPS6 (pSer235-236)
32 -
RPS6 (Total)
15 -
4E-BP1 (pSer65)
15 -
4E-BP1 (Total)

Figure S4. IRF5 isoforms increase mRNA translation rates independently of mTOR activity. A-C, IRF5(-) MEFs, and MEFs expressing HA-tagged IRF5 V5 (A-C), HA-tagged IRF5 V1 (A), V3/V4 (B) or V2/V6 (C) were incubated in Complete Media or EBSS for 2 h. MEFs were incubated with puromycin (100 μg/mL) for 1h prior to harvest and then subjected to immunoblotting with anti-puromycin antibody. (D) IRF5 (-) and MEFs expressing indicated HA-tagged IRF5 variants were incubated in Ctrl or EBSS medium for 2 h then analyzed by immunoblotting as indicated. (A-D) All results are representative of n≥2 independent experiments.

Figure S5.

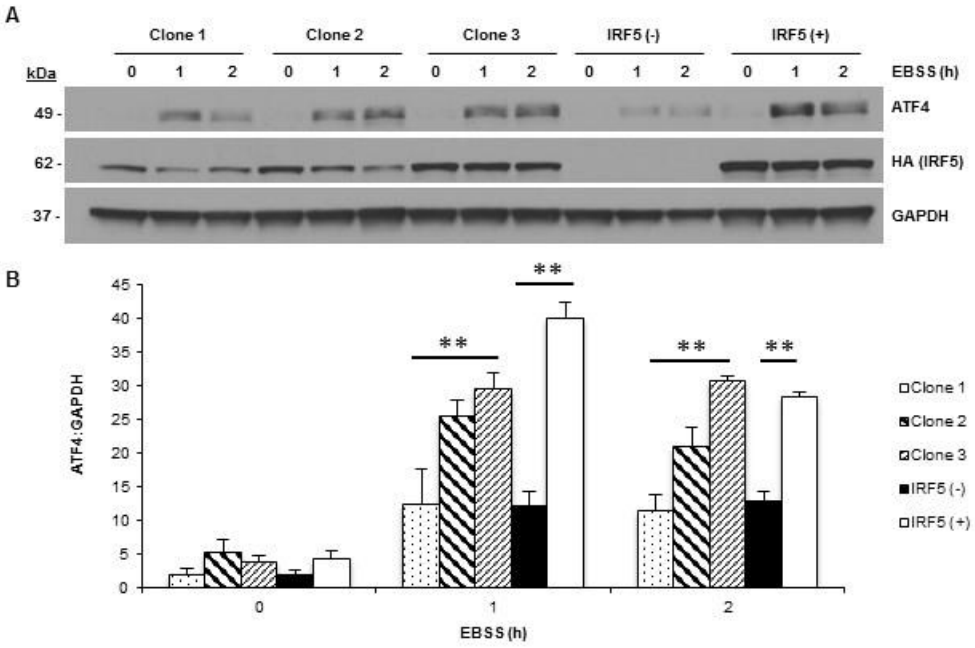

Figure S5. IRF5 and ATF4 expression are positively correlated. A and **B**, IRF5(-) MEFs and multiple IRF5(+) MEF clones each expressing different levels of IRF5 were incubated in EBSS as indicated and then subjected to immunoblotting (A). Quantitative analysis of data shown in A. Data is from n=3 independent experiments and is expressed as the ATF4-to-GAPDH ratio (B).

Figure S6.

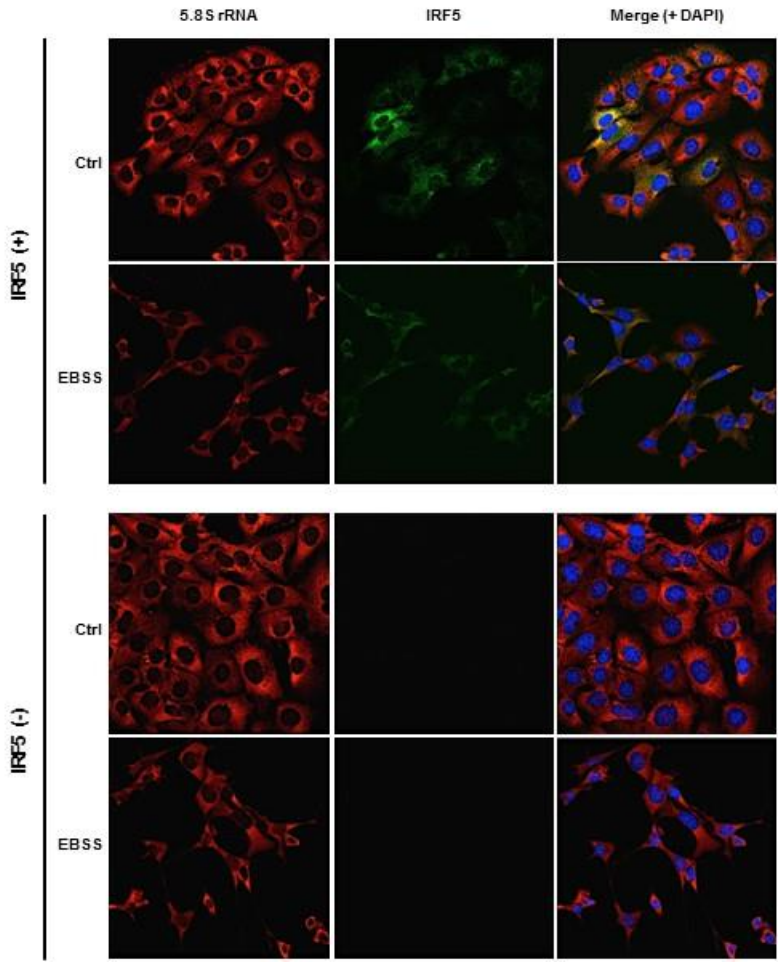

Figure S6. IRF5 remains localized within the cytoplasmic compartment following culture in EBSS. IRF5(-) and IRF5(+) MEFs were incubated in Culture Media or EBSS for 2 h and then subjected to immunofluorescence staining with 5.8S rRNA antibody (red), HA antibody (green), and DAPI (blue). Results are representative n=3 independent experiments.